RED YEASTS.

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(WITH PLATES II AND III)

THE so-called red yeasts are what are known as "wild" yeasts, and occur usually in the air. Though several species of these yeasts are known, but two, *S. rosaceus* and *S. glutinis*, are described and named. *S. rosaceus* has been studied by Hansen, but as no spores were formed during its development, he excluded it from the true Saccharomycetes. The same exclusion should apply to *S. glutinis*, for in this species, also, spore formation is unknown. In an article of recent publication, Swan[1] describes a red yeast obtained from a stale egg, which formed spores, although under somewhat extraordinary circumstances. Instead of forming spores on the gypsum block or on filter paper in the ordinary way and at 25° C. or 15° C., Swan obtained the spores in growths on wort gelatine, in the light, and at a temperature between 5°–10° C., the first indications of spores appearing in ten to fourteen days. He does not state whether or not he used ordinary conditions before resorting to these somewhat peculiar ones. The article is accompanied by photographs which show unmistakable spore formation in the yeast cells.

Of three red yeasts which the writers obtained from the air of the laboratory, and designated numbers 1, 2, 3, number 1 was at first supposed to be the same form that Swan had examined, but with subsequent work it proved to be different, varying in certain characteristics, notably the manner of growth of the colonies and the spore formation. The colonies show considerable growth in three days, and are of a dull pink color. They are not raised from the surface of the gelatine as were those of Swan, but are round and flat and have smooth edges (*Plate II*,

[1] Central. f. Bakt. u. Parasitenk. **2**: 1–11. 1896.

A). As the colonies age their color becomes much deeper, until in old growths the color is a vivid red. The color is not affected by the ordinary acids or alkalies. The cells show no color under the microscope, are oval or round, the average size being 8.6 $\mu \times 6.4\ \mu$. During vigorous growth the cells have from one to three vacuoles, the protoplasm shows very little granulation, and no chain formation takes place. The cells form but one bud at a time, and the bud separates from the mother cell before a second bud is formed. The following table gives the characteristics of growth under various conditions:

NUMBER 1.

No.	Kind	Medium	Temp.	Color	Manner of growth	Age days
1	Streak	10% Wort-gelatine	21° C.	Red	Abundant, oily in appearance, smooth edges	21
2	"	"	7° C.	Pale pink	Slight growth, oily, smooth edges	21
3	"	"	17° C.	Red	Same as no. 1	21
4	Stab	"	21° C.	Red	Growth on surface only, oily, smooth edges	21
5	"	"	7° C.	Pale pink	Slight growth, other respects like no. 4	21
6	"	Agar	21° C.	Deep red	Growth very heavy	21
7	"	Starch paste	21° C.	Pale pink	Slight growth on surface.	14
8		Wort	21° C.	Pink	Heavy sediment, liquid turbid, no gas, ring at surface	4
9		Bouillon	21° C.	Pale pink	Slight sediment, liquid cl'r, thin granular film	4
10		5% Dextrose	21° C.	Red	Heavy sediment, liquid cl'r, no gas	18
11		"	7° C.	Faint pink	Slight growth	47
12		Glucose	21° C.	Pink	Like no. 10, but less sediment	18
13		"	7° C.	Faint pink	Slight growth	47
14		Sucrose	21° C.	Red	Most abundant growth of sugar solutions, liquid slightly turbid, thin film, heavy ring at surface	18
15		Dist. water	21° C.	Very faint pink	Slight sediment, cells empty	11
16		Lactose	21° C.	Pink	Next to 14 in growth, film	18
17	Ferm. tube	Pasteur sol. with sugar	21° C.	Pink	Sediment in bend of tube, no gas	30

The foregoing table gives a general idea of the conditions used for the growth of number 1. It was found that the yeast

in a stab culture in gelatine or agar gave no growth along the needle track, the color being entirely at the surface, this showing it to be aerobic. Upon 10 per cent. wort gelatine and nutrient agar the best growths were obtained, and in the light. Various temperatures were tested, that between 17°–21° C. being found the best. At one time twenty-four varieties of yeast were grown at that temperature, and of the twenty-four number 1 gave the most vigorous growth. The color shows sooner and is stronger when the growth is made in the light. Inoculations in beer wort give a very appreciable growth in two days, the liquid becoming turbid, and a sediment forming. No film is formed, but in about a week a thin ring appears around the surface of the liquid; this ring increases in size and deepens in color with the age of the culture. Fair growths, as indicated by the sediment, were obtained in bouillon and in distilled water; in the latter, however, little color was present. Cultures in sugar solutions—sucrose, dextrose, maltose, lactose, and Pasteur solution with sugar—were made, and all of them offer good media for growth, though varying considerably. Remarkably large growths were obtained in lactose and sucrose, these being characterized by heavy sediment of a deep red color, heavy surface ring, and turbid liquid. A peculiar phase of the growth in sucrose is that the cells formed spore-like bodies. In no other of the sugars did this occur. Cultures in the sugars and in wort were made in fermentation tubes, but no gas was formed, though the cultures were kept for two months.

Spore formation was tested in the usual way, vigorously growing cells being placed on gypsum blocks. The blocks were kept under various conditions of light and temperature, with the result that at from 17°–21° C., and in the dark, granulations appeared in cells, these afterward forming spore-like bodies. The granulations first appeared in about forty hours, though it was about four days before the spore-like bodies were formed. These bodies are round and highly refractive, averaging 3.3μ in size, but developed no spore wall that could be distinguished. Being doubtful as to the true nature of these bodies, and think-

ing that probably they might be fat globules, the cells were treated with ether, alcohol, osmic acid, potash, and alkanet, all of which failed to change the bodies. Some of the cells were then placed in a drop of wort in a moist chamber at 25° C. to determine their method of germination. The chamber was placed under the microscope, so that any changes might be observed. In sixteen hours the bodies had become swollen, and they passed gradually from the swollen condition to the ordinary condition of the protoplasm in a vegetative cell. The spore-like bodies formed in the sediment and film of a 5 per cent. sucrose solution were tested in the same way, with like results. The conclusion which can be drawn from the behavior of this organism is that it is not a true yeast, but that the cells have the power of forming bodies which function as spores in preserving the organism from extinction under adverse conditions.

Plate III, B, shows the cells from a gypsum block with the contained bodies. Growths on blocks kept in the dark and at 7° C. developed but slight color, while blocks kept at 21° C. developed quite a marked color, both in the dark and in the light, thus indicating that a certain amount of heat is necessary for color development.

NUMBER 2.

No.	Kind	Medium	Temp.	Color	Manner of growth	Age days
1	Streak	Wort-gelatine	21° C.	Salmon pink	Oily, smooth edges, abundant	20
2	"	Agar	"	"	Oily, smooth edges, more abundant than in no. 1	20
3	Stab	Wort-gelatine	"	"	Slight growth along needle track	20
4	"	Agar	"	"	Slight growth along needle track	20
5	"	Starch	"	"	Oily growth on surface	20
6	Ferm. tube	Pasteur sol. with sugar	"	Pink	Sediment in bed, no gas	30
7		Bouillon	"	Pale pink	Heavy sediment, liquid turbid	18
8		Dextrose	"	"	Heavy sedim't, liquid clear	20
9		Lactose	"	"	" " " "	20
10		Glucose	"	"	" " " "	20
11		Sucrose	"	"	" " " "	20

The colonies of no. 2 are somewhat indistinct in color until they are about ten days old, when they have a yellowish-red color. Their general appearance is very nearly the same as those of no. 1, but when magnified, differences show, as seen in *Pl. II, C*. The plate cultures of the three forms, from which the photographs were taken, were made at the same time and under similar conditions. The photographs were also taken at the same time. The cells of no. 2 are round, averaging $4 \times 4\mu$ in size, and bud frequently. In stab cultures a slight growth appears along the needle track. In streak cultures, as also in colonies and the top of stab cultures, the growth is very oily in appearance, and possesses little or no color at first, though this develops as the culture ages, until in very old ones the color is quite marked. As in no. 1, the color is unaffected by the ordinary acids and alkalies. In wort and sugar solutions the growth is fairly vigorous, though not so much so as in no. 1. No film is formed, nor is any gas developed. No spores or spore-like bodies are formed either on gypsum blocks or in any of the growths, the only change in the cells, when placed under conditions for spore formation, being a granulation of the protoplasm.

NUMBER 3.

No.	Kind	Medium	Temp.	Color	Manner of growth	Age days
1	Stab	Agar	21° C.	Faint pink	Slight growth along needle track, surface growth dry, granular, abundant	14
2	Stab	Gelatine	"	Pink	Like no. 1	14
3	Streak	Agar	"	Pink	Growth abundant, dry, floury, most of color in center in heaviest growth, edges almost white	20
4	Streak	Gelatine	"	Pink	Like no. 3	20
5	Stab	Starch	"	Faint pink	Slight growth on surface	14
6		Wort	"	Pink	Turbid after a week, granular film, sediment heavy, no gas	20
7		Lactose	"	Faint pink	Slight sediment, no film, no gas	14
8		Dextrose	"	"	" " " "	14
9		Glucose	"	"	" " " "	14
10		Sucrose	"	"	" " " "	14

In no. 3 the colonies are entirely distinct from those of nos. 1 and 2; they stand out from the gelatine as feathery masses with very irregular edges, and of a salmon pink color. *Pl. II, D* shows the peculiar appearance very well. At first sight the colonies look somewhat like a mold, but upon closer inspection they show no ramifying filaments. This peculiar appearance of the colonies is due to the form of the cells, and their remarkable method of growth. A few of the cells have the regular round or oval shape of the ordinary yeast cell, but the remainder have irregular, swollen, or filamentous forms, resembling the involution forms of bacteria. *Pl. III, E* is from a vigorous growth on gelatine. These forms, with about the same length of filament are constant for young growths on solid media, also for the first colonies obtained from exposure to the air. In old growths the form varies, the filaments becoming much longer, and more branched; this lengthening and branching also takes place in cells grown in liquid media. *Pl. III, F* is a photograph from an old growth. *Pl. III, G* is a photograph from a culture in wort. The cells, though branching in a manner which resembles a mold, never develop a mycelium. The cells vary in size from 1.5μ to 3.13μ for the short diameter, and 3μ to 29μ for the long diameter. A fairly vigorous growth is obtained at room temperature on agar and wort gelatine. On both of these the growth is dry and floury in appearance, of a light pink color, and in stab cultures most of the growth is on the surface, only a slight growth appearing along the needle track. In wort it takes four or five days usually before there is sufficient growth to make the liquid turbid; then a film, that is granular, appears on the surface. There is no fermentation in wort or in any of the sugar solutions. In starch paste there is a slight pinkish growth on the surface, but very slight, however, compared with the growth on gelatine and agar.

Of the three forms studied not one of them is a true Saccharomyces. They resemble the Saccharomycetes in their appearance, both microscopically, and in gross growth, but they are unable to form true ascospores. No. 1 appears to be

a form between the Saccharomycetes and the Torulae, in that it forms spore-like bodies under the ordinary conditions for spore formation, but these bodies are not true spores, as they neither form the spore wall, nor bud in germinating. It resembles *S. rosaceus*, as heretofore described — excluding Swan's description — in appearance of colonies, and in not forming chains, but differs from it in forming spore-like bodies, and in size; *S. rosaceus* is 9–10μ in diameter, a larger form than no. 1. No such form is described in any of the standard works upon the Saccharomycetes, which would indicate that it is a new species.

No. 2 is undoubtedly *S. glutinis*, in spite of the fact that it is not of the same size as the *S. glutinis* as described in some of the later works. No. 2 answers the description in every other particular. An error seems to have crept into the description of this species in regard to its size, the other characters being practically the same in all the descriptions. Cohn[2] in 1875 gives 4–5μ as the size, while Winter[3] in 1881 gives it as 5–11×4μ, Schröter,[4] in 1893, gives 5–6×4–5μ, and Saccardo,[5] in 1897, gives the same size as Winter, 5–11×4μ. Jelliffe[6] in a list of Saccharomycetes occurring in the air gives *S. glutinis* among the number, but as he gives no description of his forms, it is impossible to tell which description he followed. The following description,[7] which was written by Schröter indicates probably the source of the error: *S. glutinis* (Fres.) F. Cohn (Zellen kugelig, 4–5μ breit) und *S. Fresenii* Schröter (Zellen ellipsoidisch bis cylindrisch, bis 11μ lang, 4–5μ breit) schlagen sich häufig aus der Luft auf Leim nieder und bilden fleisch-bis rosenrote Schleimhäufchen.

No. 3 from its peculiar appearance would seem to be easy of identification. In general appearance it is very like *Mycoderma Humuli* as described by Lasché,[8] but it is unlike in that *M. Hum-*

[2] Beiträge zur Biologie der Pflanzen 1: 187-8. 1875.
[3] Krytogamen-Flora von Deutschland 1: 71. 1881.
[4] Krytogamen-Flora von Schlesien 3: 207. 1893.
[5] Sylloge Fungorum 8: 919. 1897. [6] Bull. Torr. Bot. Club 24: 480-1. 1897.
[7] ENGLER and PRANTL, Pflanzenfamilien 1[1]: 154. 1894.
[8] Zwei rothe Mycoderma-Arten, Der Braumeister 5: 278-82. 1892.

uli liquefies the gelatine very quickly, sinking to the bottom as a red mass, forms a ring at the surface, and decomposes the liquefied gelatin, generating a foul odor, whereas no. 3 neither liquefies gelatin quickly nor gives off a foul odor in any stage of its culture. The indications are that no. 3 is a new species of Mycoderma.

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BIBLIOGRAPHY.

BAY, J. C.: Is the red Torula a true Saccharomyces? Centralb. f. Bakt. u. Parasitenk. **2**: 259-61. 1896.

COHN, F.: Beiträge zur Biologie der Pflanzen **1**: 187-8. 1875.

CROOKSHANK, E. M.: Bacteriology and infective diseases. 1897.

ENGLER and PRANTL,: Pflanzenfamilien 1^{r}: 154. 1894.

FRAENKEL, C.: Bacteriology. 1897.

GROVE, W. B.: The bacteria and yeast fungi, 1884.

JELLIFFE, S. E.: Bull. Torr. Bot. Club **24**: 480. 1897.

JÖRGENSEN, A.: Micro-organisms and fermentation, 1893.

LASCHÉ, A.: Der Braumeister **5**: 278-82. 1892.

NOVY, F. G.: Bacteriology. 1894.

SACCARDO, P. A.: Sylloge Fungorum **8**: 919. 1897.

SCHRÖTER, J.: Kryptogamen-Flora von Schlesien **3**: 207. 1893.

STERNBERG, G. M.: Manual of Bacteriology. 1893.

SWAN, A. P.: Centralb. f. Bakt. u. Parasitenk. **2**: 1-11. 1896.

WINTER, G.: Kryptogamen Flora von Deutschland **1**: 71. 1881.

WOODHEAD, G. S.: Bacteria and their products 111.

EXPLANATION OF PLATES II AND III.

PLATE II.

A. Colonies of no. 1 grown on 10% wort gelatine. ×100.

C. Colonies of no. 2 grown on 10% wort gelatine. ×100.

D. Colonies of no. 3 grown on 10% wort gelatine. ×100.

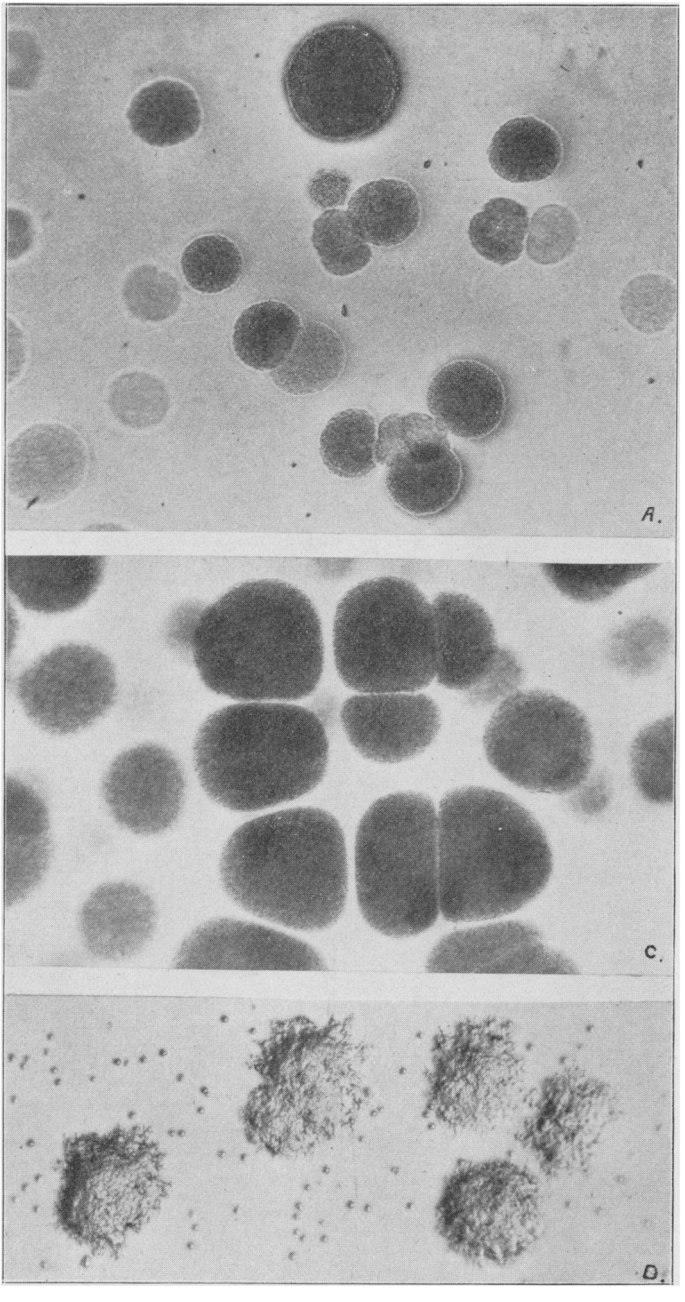
PLATE III.

B. Spore-like bodies, no. 1, grown on gypsum plates. ×495.

E. Cells of no. 3 grown on 10% wort gelatine. ×495.

F. Cells of no. 3 from old growth on agar. ×495.

G. Cells of no. 3 grown in wort. ×495.



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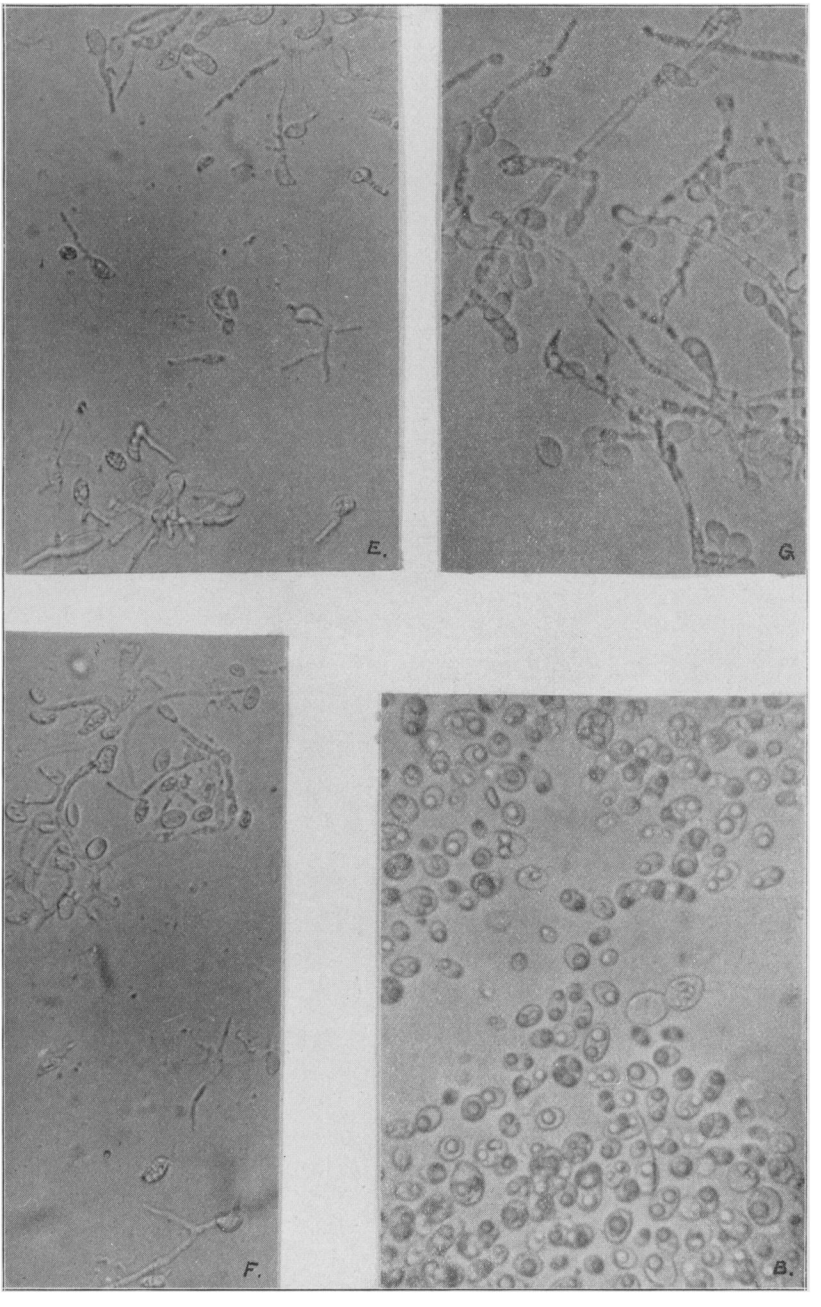

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